

Claims

1. A reconstituted cell-free translation system comprising translation factors and tRNA species capable of translating exogeneously added mRNA(s) with highly selective incorporation at each codon to form a peptide product, or a peptidomimetic product when the system includes one or more of the tRNA species charged with a non-naturally occurring amino acid or amino acid analog.
2. The translation system of claim 1 for generating a peptide product, which preparation is substantially free of the translation factors EF-P, W, W2 or rescue.
3. The translation system of claim 1 for generating a peptidomimetic product, including one or more of the tRNA species charged with a non-naturally occurring amino acid or amino acid analog that is incorporated into the backbone of the peptidomimetic product.
4. The translation system of claim 2 for generating a peptidomimetic product, which preparation is substantially free of the translation factors EF-P, W, W2 or rescue.
5. The translation system of claim 2, wherein the amino acid analog is selected from the group consisting of  $\beta$ -cyanoalanine, canavanine, djenkolic acid, norleucine, 3-phosphoserine, homoserine, dihydroxyphenylalanine, 5-hydroxytryptophan, 1-methylhistidine, 3-methylhistidine, allyl glycine (or its alkyne derivative), O-methyl-serine, biotinyl-lysine, biotinyl-cysteine (or other biotin-labelled amino acids) cyclohexylalanine, homoglutamate, D-alanine (or other D-amino acids), N-methyl glycine (or other N-methyl amino acids), epsilon-N-methyl-lysine, and radioisotope derivatives of the 21 natural amino acids or unnatural amino acids.
6. The translation system of claim 1, further including one or more exogeneously added mRNA species.
7. A cell-free translation system comprising translation factors and tRNA species capable of translating exogeneously added mRNA(s) to form a peptidomimetic product, which preparation

- 5 (a) lacks one or more active wild-type amino acyl tRNAs and ability to synthesize said wild-type amino acyl tRNA,
- (b) includes at least one exogenous amino acyl tRNA charged with a non-natural amino acid species or amino acid analog, replacing said inactive tRNA species.
8. The translation system of claim 7, including a plurality of different mRNA species.
- 10 9. A kit comprising: (a) components of the translation system of claim 1, which can be admixed to form the reconstituted cell-free translation system capable of translating exogenously added RNA to form a peptide or peptidomimetic; (b) instructions associated there with.
- 15 10. A kit comprising: (a) components of the translation system of claim 7, which can be admixed to form the cell-free translation system capable of translating exogenously added RNA to form a peptidomimetic; (b) instructions associated there with.
- 20 11. A method for generating a peptide or peptidomimetic comprising:
- (a) providing a cell-free translation system of claim 1;
- (b) contacting the translation system with one or more exogenous mRNA species;
- 25 (c) isolating and/or identifying peptide or peptidomimetic products of the translation system.
12. The method of claim 11, wherein one or more of the tRNA species of the translation system is charged with a non-naturally occurring amino acid or amino acid analog that is incorporated into the backbone of a peptidomimetic product.
- 30 13. The method of claim 11, wherein the method is carried out on a library of at least 100 different RNA species, and peptide or peptidomimetic products are identified or isolated from the translation system based on catalytic or binding activity.
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14. The method of claim 11, wherein the mRNA is generated by in vitro transcription in the translation system.
15. The method of claim 11, wherein the peptide or peptidomimetic products are formed as a covalent adduct of the exogenous mRNA by which said products are encoded.
16. The method of claim 11, wherein the translation system is contacted with a library of different exogenous mRNA species to generate variegated population of peptides or peptidomimetics products of at least  $10^3$  different sequences.
17. The method of claim 16, wherein at least  $10^8$  different sequences are produced.
18. A peptide or peptidomimetic identified or synthesized by the method of claim 11.
19. A method for generating a peptidomimetic comprising:
- (a) providing a cell-free translation system of claim 7;
  - (b) contacting the translation system with one or more exogenous mRNA species;
  - (c) isolating and/or identifying peptidomimetic products of the translation system.
20. A method for conducting a drug discovery business, comprising:
- (a) by the method of claim 11, identifying peptide or peptidomimetic products of the translation system having a desired biological activity;
  - (b) validating the biological activity of the peptide or peptidomimetic products identified in step (a) by further *in vitro* or *in vivo* assay;
  - (c) conducting therapeutic profiling of products validated in step (b), or further analogs thereof, for efficacy and toxicity in animals; and
  - (d) formulating a pharmaceutical preparation including one or more products identified in step (c) as having an acceptable therapeutic profile.
21. The method of claim 20, including an additional step of establishing a distribution system for distributing the pharmaceutical preparation for sale, and

may optionally include establishing a sales group for marketing the pharmaceutical preparation.

22. A method for conducting a drug discovery business, comprising:

- 5 (a) by the method of claim 11, identifying peptide or peptidomimetic products of the translation system having a desired biological activity;
- (b) validating the biological activity of the peptide or peptidomimetic products identified in step (a) by further in vitro or in vivo assay;
- 10 (c) (optionally) conducting therapeutic profiling of products validated in step (b), or further analogs thereof, for efficacy and toxicity in animals; and
- (d). licensing, to a third party, the rights for further drug development of validated products.

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